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Classifying pancreatic cancer using gene expression profiling

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Abstract

Despite some advances in our understanding of the molecular characteristics of pancreatic cancer, much more progress is needed. In a new study, RNA profiling of pancreatic cancers was used to identify gene signatures of tumour cells and stromal cells to help predict patient outcomes.

Pancreatic ductal adenocarcinoma (PDAC) has an overall 5-year survival of ~5% and is projected to become the second most common cause of cancer death in the USA after 2020.¹ Although chemotherapy with FOLFIRINOX and the combination of gemcitabine and paclitaxel have improved the outcomes of patients with pancreatic cancer, better therapies are still desperately needed. As mutations in genes and abnormal signalling pathways in human cancer can predict the response to certain cancer therapies, considerable interest exists in determining the genes mutated in pancreatic cancer that are ‘actionable’ and how to best identify such mutations in patient samples. For example, mutations in *BRCA2* or other genes in the BRCA pathway, which affect a small percentage of pancreatic cancers, predict responses to PARP (poly [ADP-ribose] polymerase) inhibitors in *BRCA1/2*-deficient cancers,² and the clinical utility of one of these drugs, olaparib, is being tested in patients with pancreatic cancer in a phase III clinical trial. Similarly, impressive improvements are evident in the survival of patients with melanoma and other cancers that respond to immune checkpoint inhibitors, and this response is closely related to cancers with a high mutational burden, such as cancers with DNA mismatch repair defects.³ Mismatch repair deficiency occurs in a small percentage of cancers of the gastrointestinal tract and other sites, including the pancreas.³ However, for most patients with pancreatic cancer, new insights into the biology of the disease will be required to achieve major improvements in their therapeutic outcome. In a new study, Moffitt *et al.*⁴ used RNA profiling to classify pancreatic cancers according to gene expression signatures from tumour cells and stroma that provided independent prognostic information about patient outcome.

In principle, gene expression analysis of pancreatic tumour samples could provide a wealth of information about pancreatic cancer biology. Many fundamental influences on pancreatic cancer growth and progression affect gene expression, including genetic alterations, epigenetic changes, tumour stromal interactions, immune and inflammatory responses,

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Competing interests

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hypoxia, and local and systemic metabolic effects. Could mRNA profiling be a way to provide more reliable information for predicting patient outcomes or response to therapy? Testing of mRNA profiles of pancreatic cancer samples has not yet been shown to be of value in clinical settings. One major challenge is the cellular complexity of the tumour mass: as noted by Moffitt *et al.*⁴ most of the cells within a pancreatic tumour are not cancer cells, but are non-neoplastic stromal fibroblasts, multiple types of immune cells, capillaries, neurons, adipocytes and pancreatic acinar, ductal and islet cells. As primary PDAC cells might represent as little as 5–10% of the tumour mass, identifying the cellular origin of transcripts detected in a pancreatic tumour sample and their biological significance is far from straightforward. Although microdissection can be used to isolate and molecularly characterize pure cancer-cell samples to identify aberrantly expressed genes, it poses its own challenges and is impractical for the routine characterization of the major cell types within the stroma.

To overcome some of these challenges, Moffitt and colleagues⁴ utilized purely digital techniques to ‘virtually microdissect’ bulk pancreatic tumour samples. To do this, the authors applied a bioinformatics approach, termed non-negative matrix factorization (NMF), to gene expression data obtained from microarray and RNA-sequencing analysis of primary and metastatic pancreatic tumour samples, normal tissues from the pancreas and other sites, and cell lines. The aim of the bioinformatics analysis was to identify mRNA signatures or exemplar genes representative of individual cell types within the tumour mass. Such an approach is particularly useful for characterizing the stromal compartment. The stromal compartment has a major influence on the tumour microenvironment and plays an important and still poorly understood part in tumour biology. Prior studies have indicated that certain stromal characteristics are prognostic,^{5,6} and the authors report that patients whose tumour samples exhibited an ‘activated’ stromal signature (enriched for *SPARC*, *WNT2*, *WNT5A*, *MMP9*, *MMP11*, and *FAP* mRNA transcripts) had a worse median survival time compared with those demonstrating a ‘normal’ signature. A more profound knowledge about the existence of fundamental differences in the stromal response to tumours might have important implications for therapies under investigation to target the stroma, such as hyaluronidase.⁷

Another signature was identified in this study, which predicted to represent tumour-cell gene expression.⁴ Patients with ‘classical’ tumours had a longer median survival time (19 months) than patients with ‘basal-like’ tumours (11 months). The prognostic differences for the stromal and tumour-cell signatures were independent of pathological predictors of outcome. A subset of the pancreatic cancer samples had mutational data available to search for associations with their gene signatures. The authors found that *KRAS* p.Gly12Asp mutations were associated with the basal-like signature, whereas *KRAS* p.Gly12Val mutations were associated with the classical signature. Tumoral loss of *SMAD4* expression was also associated with basal-like gene signatures. Prior studies have found that loss of *SMAD4*, which is inactivated by intragenic mutation or homozygous deletion in ~50% of PDACs, is a predictor of poor outcome and increased probability of developing metastatic disease.⁸ The authors found that *GATA6* was overexpressed and mucin expression patterns were enriched within the classical tumour subtype, which might partly reflect the extent of tumour differentiation. Moffitt *et al.*⁴ cross-validated the prognostic differences between their

‘classic versus basal’ tumour signature by showing that a similarly basal-like breast and bladder cancer signature was equally prognostic in these tumour types. Interestingly, pancreatic cancers with basal-like gene signatures had faster growth rates in mouse xenografts, whereas cancers with activated stromal gene signatures had higher graft success rates, supporting the notion that important biological differences are represented by these RNA-based tumour and stroma classifications.

Despite its strengths, one caveat to the NMF analysis is that it does not take losses of gene expression into account. Tumoural loss of gene expression arises not only through genetic mechanisms, such as homozygous deletion of genes, but also through epigenetic silencing.⁹ The detection of these losses is not possible without isolating pure populations of cells using actual microdissection or other techniques such as FACS analysis.¹⁰ Similarly, potentially important changes in the expression of genes from a tumour cell that might also be expressed by other cells within the tumour microenvironment are not adequately determined in a virtual microdissection analysis. However, it is encouraging that—despite the many sources of potential variability in gene expression within pancreatic bulk tumour samples—the gene expression signatures yielded clear prognostic information.

An interesting question that arises from the present study is which biological mechanisms might be responsible for the gene expression signatures of tumour cells and the stroma. Ultimately, whether or not the expression signatures of resected pancreatic tumours identified in this study will have clinical value needs further study. One challenge in this respect is the difficulty in obtaining samples representative of a pancreatic tumour mass from fine needle aspirates or biopsies. Beyond the prognostic signatures identified, improving management decisions for our patients with pancreatic cancer will require biomarkers that can reliably predict responses to existing and emerging therapies (Box 1).

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Box 1**Clinical value of PDAC biomarkers****Precision medicine targets**

- Predicting the response to targeted therapy; for example, to PARP inhibitors: *BRCA2* mutations and *BRCA*-like cancer phenotypes (*ATM* or *ARID1A* mutations)
- Predicting the response to immune checkpoint therapy (microsatellite instability?)

Stromal phenotyping*

- 'Active' versus 'normal' stroma
- The nature of the inflammatory infiltrate
- The stromal barrier to drug delivery

Tumour phenotyping*

- Mutation profiling for other targetable and/or actionable mutations
- Identifying relevant tumour subclones (genetic tumour heterogeneity)
- RNA profiles
- Proteome or kinome profiling
- Epigenetics
- Metabolomics

Strategies to overcome high stromal background

- Deep sequencing
- Microdissection
- Flow cytometry
- Virtual microdissection

*With the possible exception of identifying precision medicine targets, the clinical value of tumour and stroma phenotyping remains to be determined. Abbreviations: ATM, ataxia telangiectasia mutated (also known as serine-protein kinase ATM); ARID1A, AT-rich interactive domain-containing protein 1A; BRCA2, breast cancer type 2 susceptibility protein; PARP, poly [ADP-ribose] polymerase.